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It is believed that the earliest type of atherosclerotic lesion is formed by binding of monocytes and T lymphocytes

(CD4<sup>+</sup> and CD8<sup>+</sup>) to the surfaces of endothelial cells in the lumen of the artery wall. These migrating cells proceed to penetrate beneath the arterial surface. The monocytes become macrophages, accumulate lipid, and become foam cells. These cells, together with the T lymphocytes, form a lesion called the fatty streak. The fatty streak subsequently develops into a fibrofatty intermediate lesion which is composed predominantly of layers of smooth muscle cells together with lipid-filled macrophages and T cells. These lesions in turn develop into complex occlusive lesions called fibrous plaques. The fibrous plaques can increase in size by projecting into the arterial lumen, and may thereby impede the flow of blood. Sudden death from myocardial infarctions can result from ruptures in the fibrous cap of the plaque, causing hemorrhage into the plaque, thrombosis and occlusion of the artery.

Current treatments for atherosclerosis include bypass grafting, endarterectomy, and angioplasty. These methods are high-risk invasive surgical procedures. Moreover, the failure rate of such treatments can often be high due to restenosis, which is thought to result from further inflammation, smooth muscle accumulation and thrombosis.

#### Summary of the Invention

It is an object of the invention to provide a safe, effective, easy and inexpensive method for treating or preventing atherosclerosis.

It is yet another object of the invention to provide a method for treating or preventing atherosclerosis which does not involve an invasive procedure.

It is yet another object of the invention to provide a simple method for treating or preventing atherosclerosis such as administering a pill, administering an injection or inserting an implant.

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The agent can be, e.g., a soluble form of at least a portion of P-selectin or the ligand or mixtures thereof; an inhibitory protein, e.g., an antibody, e.g., a polyclonal or a monoclonal antibody, against at least a portion of P-selectin or the ligand or mixtures thereof; an inhibitory peptide, e.g., consisting of at least a portion of one of the binding sites on P-selectin or the ligand or mixtures thereof; an inhibitory carbohydrate, e.g., sialyl-Lewis x or

its analogs, sialyl-Lewis a or its analogs, heparin oligosaccharides or carbohydrates containing 2,6 sialic acids; an inhibitory glycoprotein, e.g., PSGL-1, 160 kD monospecific P-selectin ligand, lysosomal membrane glycoprotein or glycoprotein containing sialyl Lewis X; an inhibitory sulfatide; analogs of P-selectin or the ligand or mixtures thereof; substances derived from natural products, e.g., snake venoms or plant extracts; inhibitors of granular release; or inhibitors of a molecule required for the synthesis, post-translational modification, or functioning of P-selectin or the ligand.

In certain embodiments, the agent inhibits interaction between P-selectin and the ligand so as to at least partially prevent formation of, or to at least partially reverse a formed, atherosclerotic fatty streak, and/or an intermediate lesion, and/or a fibrous plaque, or so as to at least partially prevent growth of an atherosclerotic lesion after a surgical procedure for preventing restenosis.

Variations of this method of this invention include administering the agent prior to formation of an atherosclerotic lesion, administering the agent subsequent to formation of an atherosclerotic lesion, and administering the agent to a human.

Another aspect of the invention is a therapeutic agent in a dosage form and concentration suitable for treating or preventing atherosclerosis in a mammal in need of such treatment, the agent being effective to inhibit interaction between P-selectin and a ligand of P-selectin.

The above and other objects, features and advantages of the present invention will be better understood from the following specification.

P-selectin is a cell surface adhesion receptor. A receptor is a transmembrane protein with three major domains. The extracellular domain has an active site on the exterior side of the membrane which recognizes and binds to a ligand. A short hydrophobic domain makes up the transmembrane portion, and an intracellular cytoplasmic domain transmits a signal to the cell that the ligand has bound to the receptor. The extracellular domain of P-selectin includes a  $\text{Ca}^{++}$ -dependent C-type lectin domain, an epidermal growth factor-like domain, and a series of consensus repeats related to those of complement-binding proteins.

P-selectin is expressed in various cells, including endothelial cells and platelets. P-selectin mediates adhesion of different types of cells to each other. For example, P-selectin typically mediates heterotypic interactions of platelets or endothelial cells with blood cells. Cells which bind to P-selectin include monocytes,

neutrophils, eosinophils, CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells and natural killer cells.

The binding of P-selectin to another cell can result from recognition of a ligand for P-selectin on that cell. By ligand is meant a moiety which binds to P-selectin, the moiety being either alone or attached to another molecule.

P-selectin ligands include carbohydrate groups, e.g., sialyl-Lewis X (Foxall et al., J. Cell Biol., 117(4): 895-902, 1992; Polley et al., Proc. Nat'l Acad. Sci., USA, 88:6224-6228, 1991) sialyl-Lewis a (Berg et al., J. Biol. Chem., 266: 14869-14875, 1991), sialyl-Lewis x pentasaccharide (Mulligan et al., Nature 364: 149-151, 1993), polylactosaminoglycan, carbohydrate containing 2,6 sialic acid (Larsen et al., J. Biol. Chem. 267: 11104-11110, 1992), Lewis x 3'-O-sulfate (Yuen et al., Biochemistry 31: 9126-9133, 1992) and heparin oligosaccharides (Nelson et al., Blood 82: 3253-3258, 1993). P-selectin ligands are also meant to include glycoproteins which contain a carbohydrate structure. For example, a P-selectin carbohydrate ligand can be linked to a mucin-like molecule. (Sako et al., Cell 75(6): 1179-1186, 1993; Linter et al., J. Biol. Chem. 269: 471-481, 1994). By mucin is meant serine- and threonine-rich proteins that are heavily O-glycosylated and have an extended structure. Other glycoprotein ligands include PSGL-1, 160 kD monospecific P-selectin ligand (Linter et al., J. Biol. Chem. 269: 471-481, 1994) and lysosomal membrane glycoproteins (Fukuda, J. Biol. Chem. 266: 21327-21332, 1991). Analogs of the above ligands which can bind to P-selectin, e.g., where fucose is replaced, e.g., by a diol group, or derivatives of the sialyl-Lewis x compounds which carry a SO<sub>3</sub><sup>-</sup> group instead of sialic acid, or contain a sialic acid in a 2,6 linkage, are also meant to be included as P-selectin ligands.

It is known that P-selectin is involved in cellular responses to inflammation resulting from injury or

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The agent of this invention can inhibit interaction between P-selectin and a ligand of P-selectin. By inhibiting interaction is meant, e.g., that P-selectin and its ligand are unable to properly bind to each other to effect proper formation of atherosclerotic lesions. Such inhibition can be

Examples of agents include soluble forms of P-selectin or the ligand, inhibitory proteins, inhibitory peptides, inhibitory carbohydrates, inhibitory glycoproteins, inhibitory glycopeptides, inhibitory sulfatides, synthetic analogs of P-selectin or the ligand, certain substances derived from natural products, inhibitors of granular release, and inhibitors of a molecule required for the synthesis or functioning of P-selectin or the ligand.

The soluble form of either P-selectin or the ligand, or a portion thereof, can compete with its cognate molecule for the binding site on the complementary molecule, and thereby reduce or eliminate binding between the membrane-bound P-selectin and the cellular ligand. The soluble form can be obtained, e.g., from purification or secretion of naturally occurring P-selectin or ligand, from recombinant P-selectin



Inhibitory proteins include, e.g., anti-P-selectin antibodies (Palabrica et al., *Nature* 359: 848-851, 1992; Mulligan et al., *J. Clin. Invest.* 90: 1600-1607, 1992; Weyrich et al., *J. Clin. Invest.* 91: 2620-2629, 1993; Winn et al., *J. Clin. Invest.* 92: 2042-2047, 1993); anti-P-selectin ligand antibodies (Sako et al., *Cell* 75(6): 1179-1186, 1993); Fab<sub>2</sub> fragments of the inhibitory antibody generated through enzymatic cleavage (Palabrica et al., *Nature* 359: 848-851, 1992); P-selectin-IgG chimeras (Mulligan et al., *Immunol.* 151: 6410-6417, 1993); and carrier proteins expressing a carbohydrate moiety recognized by P-selectin. The antibodies can be directed against P-selectin or the ligand, or a subunit or fragment thereof. Both polyclonal and monoclonal antibodies can be used in this invention. Preferably, monoclonal antibodies are used. Most preferably, the antibodies have a constant region derived from a human antibody and a variable region derived from an inhibitory mouse monoclonal antibody. Antibodies to human P-selectin are described in Palabrica et al., *Nature* 359: 848-851, 1992; Stone and Wagner, *J.C.I.*, 92: 804-813, 1993; and to mouse P-selectin are described in Mayadas et al., *Cell*, 74: 541-554, 1993. Antibodies to human ligand are described in Sako et al., *Cell* 75(6): 1179-1186, 1993. Antibodies that are commercially available against human P-selectin include clone AC1.2 monoclonal from Becton Dickinson, San Jose, CA.

An inhibitory peptide can, e.g., bind to a binding site on the P-selectin ligand so that interaction as by binding of P-selectin to the ligand is reduced or eliminated. The inhibitory peptide can be, e.g., the same, or a portion of, the primary binding site of P-selectin, (Geng et al., J. Biol. Chem., 266: 22313-22318, 1991, or it can be from a different binding site. Inhibitory peptides include, e.g., peptides or fragments thereof which normally bind to P-selectin ligand, synthetic peptides and recombinant peptides. In another embodiment, an inhibitory peptide can bind to a molecule other than P-selectin or its ligand, and thereby interfere with the binding of P-selectin to its ligand because the molecule is either directly or indirectly involved in effecting the synthesis and/or functioning of P-selectin and/or its ligand.

Inhibitory carbohydrates include oligosaccharides containing sialyl-Lewis a or sialyl-Lewis x or related structures or analogs, carbohydrates containing 2,6 sialic acid, heparin fractions depleted of anti-coagulant activity, heparin oligosaccharides, e.g., heparin tetrasaccharides or low weight heparin, and other sulfated polysaccharides. Inhibitory carbohydrates are described in Nelson et al., Blood 82: 3253-3258, 1993; Mulligan et al., Nature 364: 149-151, 1993; Ball et al., J. Am. Chem. Soc. 114: 5449-5451, 1992; De Frees et al., J. Am. Chem. Soc. 115: 7549-7550, 1993. Inhibitory carbohydrates that are commercially available include, e.g., 3'-sialyl-Lewis x, 3'-sialyl-Lewis a, lacto-N-fucopentose III and 3'-sialyl-3-fucosyllactose, from Oxford GlycoSystems, Rosedale, NY.

Inhibitory glycoproteins, e.g., PSGL-1, 160 kD monospecific P-selectin ligand, lysosomal membrane glycoproteins, glycoprotein containing sialyl-Lewis x, and inhibitory sulfatides (Suzuki et al., Biochem. Biophys. Res. Commun. 190: 426-434, 1993; Todderud et al., J. Leuk. Biol.

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52: 85-88, 1992) that inhibit P-selectin interaction with its ligand can also be used in this invention.

Synthetic analogs or mimetics of P-selectin or the ligand also can serve as agents. P-selectin analogs or mimetics are substances which resemble in shape and/or charge distribution P-selectin. An analog of at least a portion of P-selectin can compete with its cognate membrane-bound P-selectin for the binding site on the ligand, and thereby reduce or eliminate binding between the membrane-bound P-selectin and the ligand. Ligand analogs or mimetics include substances which resemble in shape and/or charge distribution the carbohydrate ligand for P-selectin. An analog of at least a portion of the ligand can compete with its cognate cellular ligand for the binding site on the P-selectin, and thereby reduce or eliminate binding between P-selectin and the cellular ligand. In certain embodiments which use a ligand analog, the sialic acid of a carbohydrate ligand is replaced with a group that increases the stability of the compound yet still retains or increases its affinity for P-selectin, e.g. a carboxyl group with an appropriate spacer. An advantage of increasing the stability is that it allows the agent to be administered orally. Sialyl-Lewis x analog with glucal in the reducing end and a bivalent sialyl-Lewis x anchored on a galactose residue via  $\beta$ -1,3- and  $\beta$ -1,6- linkages also inhibit P-selectin binding (DeFrees et al., J. Am. Chem. Soc., 115: 7549-7550, 1993).

Agents are also meant to include substances derived from natural products, such as snake venoms and plant extracts, that inhibit P-selectin interaction with its ligand. Such substances can inhibit this interaction directly or indirectly, e.g., through specific proteolytic cleavage or other modification of P-selectin or its ligand.

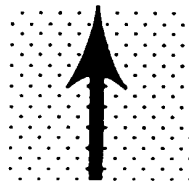
An inhibitor of granular release also interferes with P-selectin expression on the cell surface, and therefore

interferes with P-selectin function. By granular release is meant the secretion by exocytosis of storage granules containing P-selectin: Weibel-Palade bodies of endothelial cells or  $\alpha$ -granules of platelets. The fusion of the granular membrane with the plasma membrane results in expression of P-selectin on the cell surface. Examples of such agents include colchicine. (Sinha and Wagner, Europ. J. Cell. Biol. 43: 377-383, 1987).

Agents also include inhibitors of a molecule that is required for synthesis, post-translational modification, or functioning of P-selectin and/or the ligand, or activators of a molecule that inhibits the synthesis or functioning of P-selectin and/or the ligand. Agents include cytokines, growth factors, hormones, signaling components, kinases, phosphatases, homeobox proteins, transcription factors, translation factors and post-translation factors or enzymes. Agents are also meant to include ionizing radiation, non-ionizing radiation, ultrasound and toxic agents which can, e.g., at least partially inactivate or destroy P-selectin and/or the ligand.

An agent is also meant to include inhibitors which are not entirely P-selectin specific. For example, an agent may inhibit other selectin interactions in addition to P-selectin interactions, e.g., L and/or E selectin interactions. Such overlapping specificity may provide additional therapeutic advantage.

Administration of the agent can be accomplished by any method which allows the agent to reach the target cells. These methods include, e.g., injection, deposition, implantation, suppositories, oral ingestion, inhalation, topical administration, or any other method of administration where access to the target cells by the agent is obtained. Injections can be, e.g., intravenous, intradermal, subcutaneous, intramuscular or intraperitoneal. Implantation includes inserting implantable drug delivery systems, e.g.,



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microspheres, hydrogels, polymeric reservoirs, cholesterol matrices, polymeric systems, e.g., matrix erosion and/or diffusion systems and non-polymeric systems, e.g., compressed, fused or partially fused pellets. Suppositories include glycerin suppositories. Oral ingestion doses can be enterically coated. Inhalation includes administering the agent with an aerosol in an inhalator, either alone or attached to a carrier that can be absorbed.

Administration of the agent can be alone or in combination with other therapeutic agents. In certain embodiments, the agent can be combined with a suitable carrier, incorporated into a liposome, or incorporated into a polymer release system.

Preferably, protein agents are administered by intravenous or intramuscular injection; peptide agents by intravenous or intramuscular injection or by glycerin suppository; carbohydrate or sulfatide agents by intravenous or intramuscular injection, or with an aerosol in an inhalator; and synthetic analog agents by intravenous or intramuscular injection, or with an aerosol in an inhalator, or orally.

In certain embodiments of the invention, the administration can be designed so as to result in sequential exposures to the agent over some time period, e.g., hours, days, weeks, months or years. This can be accomplished by repeated administrations of the agent by one of the methods described above, or alternatively, by a controlled release delivery system in which the agent is delivered to the mammal over a prolonged period without repeated administrations. By a controlled release delivery system is meant that total release of the agent does not occur immediately upon administration, but rather is delayed for some time period. Release can occur in bursts or it can occur gradually and continuously. Administration of such a system can be, e.g.,

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Examples of systems in which release occurs in bursts include, e.g., systems in which the agent is entrapped in liposomes which are encapsulated in a polymer matrix, the liposomes being sensitive to a specific stimuli, e.g., temperature, pH, light or a degrading enzyme, and systems in which the agent is encapsulated by an ionically-coated microcapsule with a microcapsule core-degrading enzyme.

The agent can be suspended in a liquid, e.g., in dissolved form or colloidal form. The liquid can be a solvent, partial solvent or non-solvent. In many cases water or an organic liquid can be used.

The agent is administered to the mammal in a therapeutically effective amount. By therapeutically effective amount is meant that amount which is capable of at least partially preventing or reversing plaque formation. A therapeutically effective amount can be determined on an

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Preferably, the concentration of an inhibitory protein, peptide, glycoprotein or glycopeptide if applied systemically, is at a dose of about 0.1 to about 500 mg/kg body weight. Most preferably the dose is about 0.1 to about 5 mg/kg. The specific concentration partially depends upon the particular inhibitory protein, glycoprotein, peptide or glycopeptide used, as some are more effective than others. Preferably, the concentration of a carbohydrate or a synthetic analog, if applied systemically is at a dose of about 0.01 to about 200 mg/kg body weight. Most preferably, the dose is about 0.1 to about 5 mg/kg. Preferably, the concentration of a sulfatide, if applied systemically is at a dose of about 1 to about 100 mg/kg body weight. Preferably, the concentration of a soluble form of P-selectin or ligand, if applied systemically is at a dose of about 1 to about 100 mg/kg body weight. Most preferably, the dose is about 1 to about 5 mg/kg. The dosage concentration of the agent that is actually administered is dependent at least in part upon the final concentration that is desired at the site of action, the method of administration, the efficacy of the particular agent, the longevity of the particular agent, and the timing of administration relative to the formation of the atherosclerotic lesion. Preferably, the dosage form is such that it does not substantially deleteriously affect the mammal. The dosage can be determined by one of ordinary skill in the art employing such factors and using no more than routine experimentation.

The agents of the invention are meant to include reversible and non-reversible agents. If an agent is reversible, the inhibition of the interaction between P-selectin and its ligand will be reversed at some point after administration of the agent ceases. A reversible agent is preferable in that it permits discontinuation of administration of the agent during periods of infection or wounds. P-selectin function is thereby restored and able to act in its inflammation-response capacity to aid in fighting infections or in wound repair.

The invention also includes a therapeutic agent in a dosage form and concentration suitable for treating or preventing atherosclerosis in a mammal in need of such treatment, the agent being effective to inhibit interaction between P-selectin and its ligand.

#### EXAMPLES

Example 1: P-Selectin-Deficient Mice Fed a High Fat Diet Have Significantly Smaller Atherosclerotic Lesions Than Wild-Type Mice

This example illustrates that P-selectin plays an important role in the formation of atherosclerotic lesions in blood vessels. Comparisons were made of atherosclerotic lesions in wild-type and P-selectin-deficient mice fed a high fat diet. The P-selectin deficient mice contain a homozygous null mutation in P-selectin and were generated by homologous recombination in embryonic stem cells as described in Mayadas et al., Cell 74: 541-554, 1993.

Age-matched female wild-type and P-selectin deficient mice were used. (C57BL and 129 mixed background; both of these strains are susceptible to aortic lesion formation upon > 14 week exposure to a high fat diet.). The mice were

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The total cholesterol levels in the blood plasma increased by comparable amounts in both P-selectin-deficient and wild-type mice. The p value is 0.46, indicating that there was no statistical difference in cholesterol levels in response to the high fat diet in the two sets of mice. The measured cholesterol value increases were similar to those reported by Paigen et al., *Atherosclerosis*, 57: 65-75, 1985.

The hearts were processed according to Paigen et al., Atherosclerosis, 68: 231-240 (1987). The heart and attached aorta were placed in 0.9% saline for 1 hour to remove erythrocytes and allow muscle relaxation. The hearts were then fixed in 10% buffered formalin and embedded in gelatin. For quantitative evaluation, the hearts were embedded in O.C.T. (optimal cooling temperature) compound, frozen and sectioned on a cryostat. Sections were discarded until reaching the junction of the heart muscle and aorta where the valve cusps become visible and the aorta is rounded. Unstained sections were regularly examined to locate the area of interest. This area of the aorta was shown previously to consistently result in lesions in C57BL/6 mice following 14 weeks exposure to the high fat diet. (Paigen et al., Atherosclerosis, 68: 231-240, 1987). Once the area was

One section on each of the odd numbered slides was assessed. Where possible, the same section on each of the five slides was used for quantitation. Thus, five sections, each 80  $\mu\text{m}$  apart, were examined. If a section on a slide was folded or damaged, then the section immediately following or preceding replaced the flawed section. The slides were coded and the examiner was unaware of the genotype of the animal from which the sections originated. The size of the lesion was quantified using an ocular micrometer (net grid with 100 squares; each square 25 x 25  $\mu\text{m}$  using 40x objective). Lesions less than 0.1 square using the 40x objective (400x magnification) were not counted. Lesions for each section were totaled. As shown in Table 1, the average size of the lesions in the P-selectin deficient mice fed a high fat diet was 3.6 times smaller than for the wild-type mice fed a high fat diet. No aortic lesions were present in wild-type or P-selectin deficient mice (one each) fed the low fat control diet.





TABLE 2

t-TEST: TWO-SAMPLE ASSUMING EQUAL VARIANCES

	<u>Wild Type</u>	<u>P-Selectin-Deficient</u>
Mean	2,212.50	618.75
Variance	8,306,760.20	2,405,408.65
Observations	50.00	40.00
Pooled Variance	5,691,388.49	
Hypothesized Mean Difference	0	
df	88	
t Stat	3.149	
P(T<=t) two-tail	0.002	

Example 2:     Treating Atherosclerosis in a Human with Sialyl  
Lewis x

This example illustrates a method for treating atherosclerosis in a human with an agent which inhibits interaction between P-selectin and its ligand. The patient is given an intramuscular injection of sialyl-Lewis x, once a day for a period of six months. (Mulligan et al., Nature 364: 149-151, 1993). The dose concentration per day is 1 mg/kg body weight. This treatment interferes with further development of atherosclerotic lesions.

Example 3:     Treating Atherosclerosis in a Human With an  
Analog of Sialyl-Lewis x

This example illustrates a method for treating atherosclerosis in a human with an agent which inhibits

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To examine whether the absence of P-selectin can influence the development of the extensive atherosclerotic lesions in FH, the P-selectin-deficient mice described in Example 1 (Mayadas et al., Cell 74:541-554 (1993)) were bred with LDL receptor-deficient mice developed through gene targeting technology described in Ishibashi et al., J. Clin. Invest., 92:883-893 (1993). The phenotype of the LDL receptor-deficient mice is remarkably similar to the phenotype of human homozygous FH when the animals are fed an atherogenic diet rich in cholesterol, saturated fat, and cholic acid (Ishibashi et al., J. Clin. Invest., 93:1885-1893 (1994)). Through the above-described breeding, a colony of mice deficient for LDL receptor and either wild-type for P-selectin or homozygous-deficient for P-selectin have been obtained. Twelve mice which are LDL receptor-deficient and wild-type for P-selectin (P-selectin-positive), and 11 mice deficient for both LDL receptor and P-selectin (P-selectin-negative), were put on an atherogenic diet for 8 weeks. Their hearts were then processed as described in Example 1. Within two weeks of the onset of the diet, their plasma cholesterol reached levels above 1,000 mg/dl, as compared to 200 mg/dl prior to the diet administration. At the time of sacrifice, a large sample of blood was collected for individual cholesterol, triglyceride and lipoprotein profile analysis. No differences were detected between the P-selectin-negative and P-selectin-positive mice. After 8 weeks on the high cholesterol diet, the mice had practically no HDL, and most of the cholesterol was in the LDL-VLDL region, in agreement with results reported by others (Ishibashi et al., J. Clin. Invest., 93:1885-1893 (1994)). Importantly, there was no difference in the total plasma cholesterol levels between the P-selectin-positive and negative mice -- both groups gave approximately 1000 mg/dl (levels comparable to those seen in human FH) (Table 3).

TABLE 3

CHOLESTEROL LEVELS IN LDLR-DEFICIENT MICE AFTER  
8 WEEKS ON HIGH-FAT DIET (mg/dl)

<u>P-Selectin Wild Type</u>	<u>P-Selectin-Deficient</u>
842	1068
1066	1053
1088	1228
1021	1076
940	1176
1241	1025
1135	795
1046	1114
926	1036
1842	1024
1438	1283
1280	

Statistics:

	<u>P-Selectin</u> <u>Wild Type</u>	<u>P-Selectin</u> <u>Deficient</u>
mean	1155.42	1079.82
standard deviation	272.09	127.94
n	12	11
p value	0.411	

The results shown in Table 3 confirmed that the diet had the desired effect on plasma cholesterol level and also that the mice were correctly genotyped as LDL receptor-deficient. As described in Example 1, five sections of the aorta in the cusps regions were assessed. The mean area of the lesion was determined and this single value for each animal (Table 4) was used for statistical analysis (Tables 5 and 6).



MEAN ATHEROSCLEROTIC LESION SIZE (mm<sup>2</sup>)  
IN LDL RECEPTOR-DEFICIENT MICE

\* indicates males

t-TEST FOR P-SELECTIN-POSITIVE MICE  
AND P-SELECTIN-NEGATIVE MICE:  
TWO-SAMPLE ASSUMING EQUAL VARIANCES

	P-Selectin Positive (total)	P-Selectin Negative (total)
Mean	0.249	0.155
Variance	0.008	0.005
Observations	12.000	11.000
Pooled Variance	0.006	
Hypothesized Mean Difference	0.000	
df	21.000	
t Stat	2.810	
P(T<=t) two-tail	0.010	

TABLE 6

t-TEST FOR P-SELECTIN-POSITIVE MALE MICE  
AND P-SELECTIN-NEGATIVE MALE MICE:  
TWO-SAMPLE ASSUMING EQUAL VARIANCES

	<u>P-Selectin Positive (males)</u>	<u>P-Selectin Negative (males)</u>
Mean	0.296	0.146
Variance	0.009	0.007
Observations	5.000	5.000
Pooled Variance	0.008	
Hypothesized Mean Difference	0.000	
df	8.000	
t Stat	2.632	
P(T<=t) two-tail	0.030	

As shown in Table 4, the mean size of the atherosclerotic lesions in the P-selectin-positive mice was very large. Despite the overwhelming size of the atherosclerotic lesions in this FH model, the absence of P-selectin caused a significant reduction in lesion size (Table 5). This result was especially notable in males, where the lesions in the P-selectin-positive mice were twice the size of those found in P-selectin-negative animals (Table 6).

Those skilled in the art will be able to ascertain, using no more than routine experimentation, many equivalents of the specific embodiments of the invention described herein. These and all other equivalents are intended to be encompassed by the following claims.

What is claimed is: